ES 221 Problem Set 4

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In the following pages, we consider the development and optimization of an antibody-drug conjugate (ADC) used to target a novel pancreatic cancer-overexpressed cell surface receptor. The target is a member of the EHR class of cell surface receptor, of which there are three types (EHR1, EHR2, EHR3). These receptors contain extracellular, transmembrane, and intracellular kinase domains and undergo heterodimerization and autophosphorylation upon ligand binding. Receptor activation is appeared to be linked to cell proliferation, differentiation, and survival. We now consider: 1) identification of an optimal molecular target; 2) selection of human antibody; 3) optimization of Fc domain; 4) optimization of linker to drug; and 5) estimation of maximum tolerated dose. We also explore manufacturing considerations based on data from three lots used to produce ADC for a phase I trial.

1 Question 1: Selection of Molecular Target from EHR Cell-Surface Proteins

Endoscopic biopsies were obtained from cancerous and nearby healthy panceratic tissue from eight patients with advanced disease. Murine monoclonal antibodies for each of the three identified EHR proteins were used to quantify binding kinetic parameters using surface plasmon resonance (SPR), in which antibody-antigen (receptor) binding interactions on the surface of a metal (with an intermediate membrane) perturb the local oscillations of conduction band electrons (plasmons) in the metal upon irradiation with light. The magnitude of these interactions can be used to estimate the density (in $molecules/\mu m^2$) of the given EHR receptor in both healthy and diseased tissue.

The important parameter in identifying an ideal molecular target is the relative expression level, the ratio of the density of molecules on diseased versus healthy tissue, ρ_{dis}/ρ_{norm}. We can compute this for each patient and each of the three EHR receptors and calculate the average value and standard deviation of the ratios as:

$$\bar{RE} = \frac{\Sigma_{i=1}^{N}(\rho_{dis,i}/\rho_{norm,i})}{N} \tag{1}$$

$$\sigma_{RE} = (\frac{\Sigma_{i=1}^{N}(RE_i - \bar{RE})^2}{N-1})^{1/2} \tag{2}$$

An alternative method for probing this ratio is to instead calculate the averages of the diseased and healthy levels and divide the two, rather than taking the average of the ratios (note the factor of $1/N$ is left out since this is common to both the numerator and denominator):

$$\bar{RE}* = \frac{\Sigma_{i=1}^{N}\rho_{dis,i}}{\Sigma_{i=1}^{N}\rho_{norm,i}} \tag{3}$$

Analyzing the experimental data yields the information in Table 1. The two methods for estimating the average

Table 1: EHR Receptor Expression Levels.

Molecule	$\bar{RE}(molecules/\mu m^2)$	$\sigma_{RE}(molecules/\mu m^2)$	$\bar{RE}*(molecules/\mu m^2)$
EHR1	28.6	19.4	24.3
EHR2	4.07	5.65	1.69
EHR3	3.66	3.29	3.57

ratio of expression levels do not differ substantially, so either will allow us to choose a candidate target. Their similarity could be indicative of correlation (i.e. that a higher expression in healthy tissue corresponds to higher expression in diseased tissue), but the correlation coefficients are not statistically significant ($p > 0.29$ for all three receptors). Larger expression ratios make for a better target, as this reduces the likelihood of the antibody targeting healthy tissue in addition to the cancer target. Of course, overall larger magnitude of expression levels in diseased tissue is also a benefit, as this provides more targets on the cancerous tissue. Luckily, EHR1 shows both of these qualities, with the largest expression ratio by either measure and the largest average average expression in cancerous tissue ($1812 molecules/\mu m^2$). While the standard deviation of the ratios is also the largest, if we consider the standard deviation normalized to the mean expression ratio, EHR1 actually has the smallest relative standard deviation. Thus, this makes it by far the best of the three molecules to serve as a target for an antibody-drug conjugate.

2 Question 2: Selection of Human IgG1 Antibody

Using the above considerations, the EHR receptor, hereafter referred to as pancreatic cancer cell marker (PCCM), was identified as a target for an antibody-drug conjugate. Three monoclonal human antibodies were engineered using phage-display technology, in which the gene for the protein of interest (i.e. the antibody) is ligated into the coat protein gene of a bacteriophage so that the protein is expressed on the viral surface and its genetic code is contained inside the genome. SPR was again employed to test bacteriophage binding to PCCM. From the magnitude and kinetics of the plasmon perturbation, the dissociation rate constant, k_d, and association rate constant, k_a, of the antibody-receptor interaction ($[EHR] + [Ab] \leftrightarrow [EHR - Ab]$) can be determined. From these, we can obtain the equilibrium dissociation constant, $K_D = k_d/k_a$, a measure of the concentration at which half of the antibodies are bound to receptors and half are free. Given the low sample size, we can estimate a 95% confidence interval using a Student's t-distribution, where the t-score is defined as:

$$t = \frac{\bar{x} - \mu}{s/\sqrt{n}} \tag{4}$$

Here, $\bar{x}$ is the sample mean, μ is the population mean, s is the sample standard deviation, and n is the number of samples. For statistical testing, this is compared to a standard value of t, $t_{\alpha,n-1}$, where α is the significance level and $n-1$ is the number of degrees of freedom. For a t-test, the null hypothesis is that the samples come from a distribution with mean μ. A two-tailed t-test uses significance levels of $\alpha/2$ both above and below 0, testing the probability that the sample mean cannot stray too far from the population mean in either direction. This is what we employ here to estimate a 95% confidence interval of the population mean, by considering $t_{0.025,7}$ and $t_{0.975,7}$. Since the inverse of the t-distribution is antisymmetric about 0, we note that $t_{0.025,7}$ = -$t_{0.975,7}$. Thus, rearranging the above expression, we get:

$$\bar{x} - t_{0.975,7} * s/\sqrt{n} \leq \mu \leq \bar{x} + t_{0.975,7} * s/\sqrt{n} \tag{5}$$

Table 2 shows the antibody dissociation constant for each of the three tested antibodies in each patient.

Table 2: Antibody Dissociation Constants for Each Patient.

Patient	$hAb116K_D(nM)$	$hAb201K_D(nM)$	$hAb302K_D(nM)$
1001	0.484	1.27	2.80
1002	0.349	0.610	1.78
1003	0.232	1.13	4.57
1004	0.429	2.72	1.70
1005	0.337	4.58	3.17
1006	0.353	1.96	4.63
1007	0.244	3.20	1.25
1008	0.250	2.38	1.61

Table 3 shows the results for the estimated mean and confidence intervals for the dissociation constant of the three candidate antibodies.

Table 3: Mean Antibody Dissociation Constants and 95% Confidence Intervals.

Antibody	$K_{D,avg}(nM)$	$LowerBound(nM)$	$UpperBound(nM)$
hAb116	0.335	0.259	0.410
hAb201	2.23	1.15	3.30
hAb302	2.69	1.57	3.81

From these results, it is clear that hAb116 is the best choice for targeting PCCM. As dissociation constant reflects the concentration at which half of the available antibodies are bound, a lower dissociation constant is indicative of stronger binding. This should ultimately improve the targeting capability of the ADC system and enhance antibody internalization and activity of the toxin intracellularly.

3 Question 3: Optimization of FcRn Binding Domain

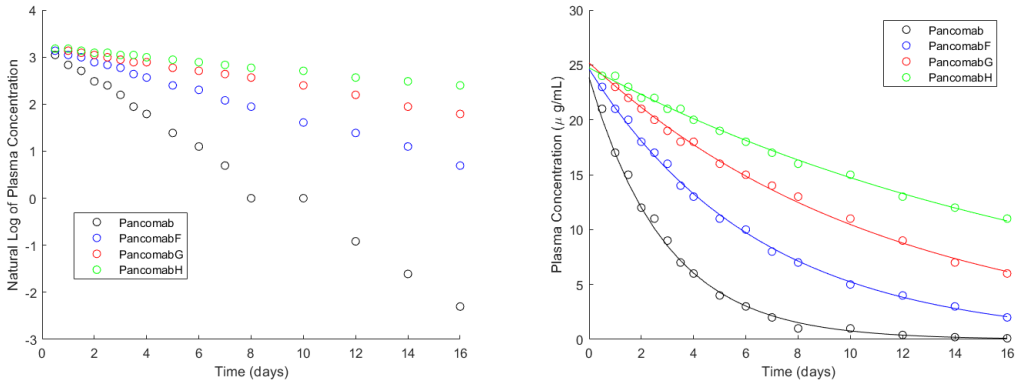
With a selected antibody, we now consider an optimization strategy to increase the pharmacokinetic half life by modifying the crystallizable fragment (Fc) of the antibody. One interaction mediated by this portion of the antibody is with the neonatal Fc receptor (FcRn), which has been shown to mediate transcytosis across cell membranes and recycling of antibodies back into the systemic circulation [1]. Thus, improving FcRn binding should extend the half-life. A pharmacokinetic analysis was performed for the unmodified antibody (hereafter referred to as Pancomab), and three modifications, PancomabF, PancomabG, and PancomabH. Plotting plasma concentration over 16 days in $n = 6$ cynomolgus monkeys on a log-linear scale versus time does not show a noticeable distribution phase, suggesting applicability of a one-compartment model of the form:

$$C_p = \frac{D}{V_d} e^{-kt} \tag{6}$$

Here, C_p is the plasma concentration of the drug, D is the bolus dose, V_d is the volume of distribution, and k is the elimination rate constant. Drug was delivered as a $1mg/kg$ bolus, and the subjects had an average weight of $5kg$, yielding an average dose of $5000\mu g$. By taking the natural logarithm of both sides, we obtain a linear equation, allowing us to fit using linear regression and obtain the remaining parameters.

$$ln(C_p) = ln(\frac{D}{V_d}) - kt \tag{7}$$

We can then estimate the pharmacokinetic half-life of the drug through the relation $t_{1/2} = ln(2)/k$. Figure 1 displays linearized experimental data showing the lack of a distribution phase (a) and the model fit to the experimental data (b). Table 4 contains the pharmacokinetic parameters from the analysis.



(a) Linearized Experimental Data.

(b) Model Fit.

Figure 1: Pharmacokinetic Analysis of Four Candidate Pancomab Antibodies.

Table 4: Pharmacokinetic Parameters of Candidate Antibodies.

Compound	$V_d(L)$	$k(d^{-1})$	$t_{1/2}(d)$
Pancomab	210	0.344	2.02
PancomabF	203	0.155	4.48
PancomabG	198	0.0879	7.89
PancomabH	202	0.0518	13.4

The volumes of distribution of all four candidate antibodies are similar, approximately $200L$, suggesting that they all quickly leave the ($2L$) plasma volume of the monkeys and enter the tissue. Thus, the half-life is the primary determinant for selecting an antibody for clinical development. A longer half-life should improve clinical efficacy, as the antibody will be present in the body for longer, and thus should be available to continue to induce death of cancer cells without the need for another dose. Thus, PancomabH is expected to be the most efficacious modification and is selected for further development.

4 Question 4: Optimization of Antibody-Toxin Linker

After optimization of the Fc region of the antibody and selection of the microtuble-inhibiting maytansinoid mertansine (DM1) as the toxin, the linker must be optimized to permit sufficient drug loading as well as activity upon endocytosis by pancreatic tumor cells. The combination of these factors (in conjunction with extravasation and retention in the tumor microenvironment) can be approximated by the *in vivo* efficacy of the ADC. Four disulfide bond linkers (Pancomab8a-d) and one thioether linker (Pancomab8e) were synthesized with an average of three DM1 molecules per antibody and the ADCs were delivered through a single IV dose of $10mg/kg$ in a xenograft mouse model with tumors derived from a human EHR-1-expressing pancreatic cancer cell line expanded *in vitro*. Tumor volume estimates ($^{\pm}20\%$) were recorded over a three-week time period with an average $n = 3$ per group. The simplest model for tumor growth is the exponential model [2]:

$$V(t) = V_0 e^{rt} \tag{8}$$

Where $V(t)$ is the tumor volume at time t, V_0 is the initial tumor volume, and r is the rate constant for tumor growth. This latter parameter should be reduced for more efficacious drugs. We can thus linearize our data and estimate this rate constant through linear regression:

$$ln(V(t)) = ln(V_0) + rt \tag{9}$$

Figure 2 shows the results from the model fits of the 5 candidate linkers and the free Pancomab antibody without any DM1 toxin. The model fit parameters are contained in Table 5. As one would expect, the slowest rate of increase

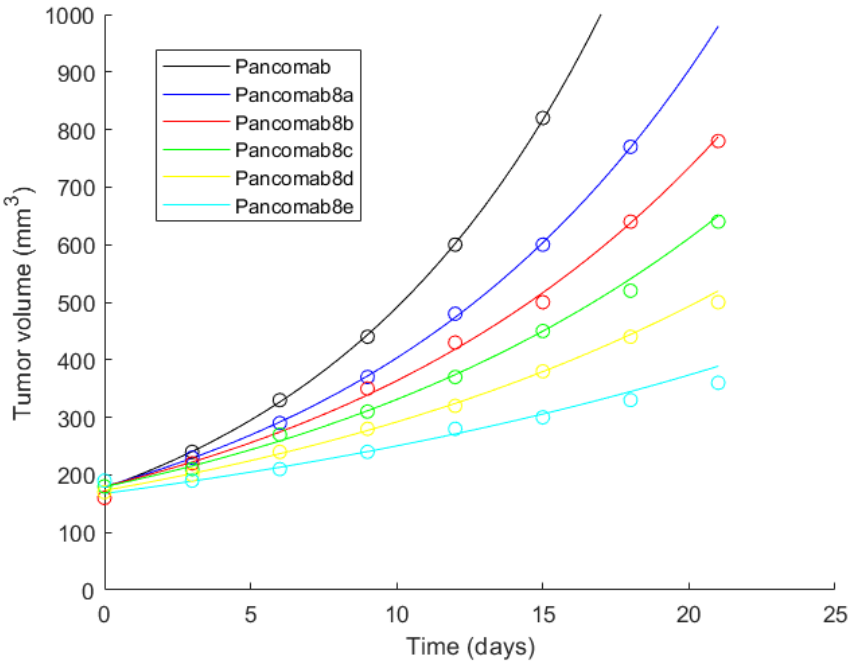


Figure 2: Exponential Fits for Tumor Growth in Response to Five Candidate ADC Linkers and Unlinked Antibody.

(i.e. the lowest value of r) corresponds to the best expected clinical efficacy. In this case, the thioether linker is optimal, as it slows the rate of increase by a factor of 0.39. Given that this is exponential growth, such a decrease is quite promising. As an example, we can consider the last time point for all six conditions have data. At 15 days into the experiment, the tumor volume of the thioether-linked DM1 ADC is 63% smaller than the undrugged control. A two-way analysis of variance (ANOVA) shows a statistically significant difference between linkers ($p = 5.44e-04$) and over time ($p = 5.62e-08$). In short, a two-way ANOVA assumes the response variable observations can be approximated by a linear model containing the mean of the distribution and the dependence on two independent variables (here, time and linker type). It then compares the sum of squares along each row and column to the sum of squared errors (i.e. deviations from the mean within each group) and compares their ratio normalized to the number of degrees of freedom to an F-statistic. A post-hoc Tukey-Kramer test reveals significantly different behavior between the untreated case and Pancomab8c-e, confirming the evidence that DM1 conjugation significantly improves

Table 5: Pharmacokinetic Parameters of Antibodies with Different Linkers.

Compound	$V_0(mm^3)$	$r(d^{-1})$
Pancomab	177	0.102
Pancomab8a	180	0.0807
Pancomab8b	180	0.0702
Pancomab8c	179	0.0613
Pancomab8d	173	0.0524
Pancomab8e	168	0.0400

efficacy. This post-hoc test compares the means of every treatment to all others. For each two-mean comparison, the difference in means is normalized by the standard error and is compared to a Studentized range distribution, $q_{\alpha,k,N-k}$ for signficance level α, number of populations k and number of degrees of freedom $N-k$. Use of a proper post-hoc test is essential to ensure that the familywise error rate (i.e. probability of at least one Type I error), does not exceed the significance level. It is worth noting that use of an ANOVA test relies on the assumption that the distributions are Gaussian (normal).

5 Question 5: Estimation of Maximum Tolerated Dose in Cynomolgus Monkeys

Prior to the initiation of a clinical trial, an estimate for the starting dose must be made, which requires identification of the maximum tolerated dose (MTD) or no observed adverse effect level (NOAEL). Primate toxicology was performed in cynomolgus monkeys to identify both acute and repeat toxic levels. The most sensitive results were found in the repeat dose case, in which 4 total doses were given every two weeks beginning at 2 weeks after initiation of the investigation. Toxicity was assessed by recording blood levels of the liver enzyme alanine aminotransferase (ALT), which catalyzes transfer of an amino group from L-alanine to α-ketoglutarate, producing pyruvate and L-glutamate.

In humans, the threshold for "normal" ALT levels varies substantially, with Prati *et al.* reporting cutoffs below 30 units per liter (U/L) for men and 19 U/L for women [3], while a review by Gowda *et al.* has an upper bound of 56 U/L[4]. Values for cynomolgus monkeys are similar to this upper bound in humans, and were previously found to have a mean value of 51.8 IU/L (equivalent to U/L) for N = 76 male monkeys and 60.0 IU/L for N = 37 female monkeys [5]. It should be noted that standard deviations were quite large (24.0 IU/L and 45.0 IU/L, respectively), so this enzyme appears to vary widely in monkeys as well. Elevated ALT and AST (aspartate aminotransferase), as well as the magnitude of AST/ALT ratio, can be indicative of specific liver disorders, including viral hepatitis and cirrhosis. In humans, ALT levels greater than 500 U/L are considered problematic, while those below 300 U/L are considered nonspecific and may be transient [4]. Mildly elevated levels (below 5 times of the normal upper bound) are relatively common, affecting 10 % of Americans [6].Therefore, while elevated ALT levels are not guaranteed to be indicative of liver disease, significant elevation relative to control groups without drug administration may suggest damage over the long term.

Four different doses were tested (2, 6, 12, and 24 mg/kg) relative to a control (0 mg/kg) group of the Pancomab8 ADC system. N=16 monkeys per group were monitored over 12 weeks, with dosing occurring on weeks 2, 4, 6, and 8. ALT levels were recorded weekly. The results are shown in Figure 3. As above, we can use a statistical method to identify the MTD. Here, rather than rely on the assumption of normality, we employ a more general Kruskal-Wallis test, which performs the same analysis as an ANOVA on the ranked data, rather than the data itself (i.e. it compares medians rather than means). The p-value that each column comes from the same distribution is $p = 1.72e-05$, so it is clear that the drug does cause significant differences in ALT levels. A post-hoc Tukey-Kramer test shows that there are no significant differences between the control and two lowest doses, the 12 mg/kg dose is only significantly different from the control, and the 24 mg/kg dose is significantly different from the control and the two lowest doses (see Figure 4). Based on this analysis, we select 12 mg/kg as the MTD as a conservative estimate. 24 mg/kg could also be justified given that the 12 mg/kg dose is not significantly different from the control group at the 0.01 significance level. However, if we restrict our analysis to just the time points over which drug is being administered (week 2 to week 9), 12 mg/kg is significantly different from the first two conditions. One final concern

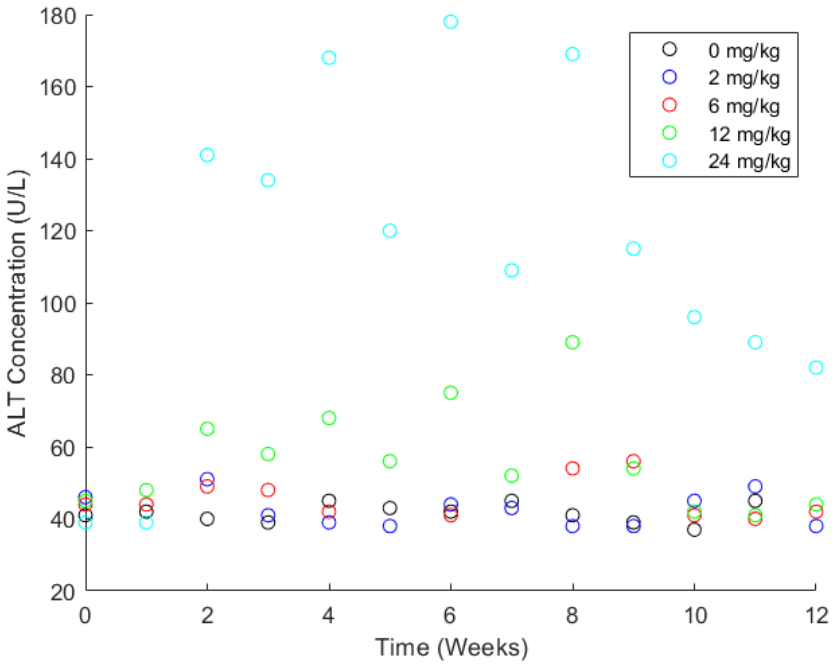


Figure 3: ALT Plasma Concentration Over Time for Different Doses.

is the potential for an increase in ALT levels over time. We check this by performing a linear regression over the same interval (week 2 to week 9) to determine if a nonzero regression slope is found that is statistically different from zero. None of the models have a regression slope coefficient that is significantly different from zero (the smallest p-value is 0.15), so this does not appear to be a problem based on the current data. We thus select a starting dose of half the MTD, i.e. $D_{start} = 6mg/kg$.

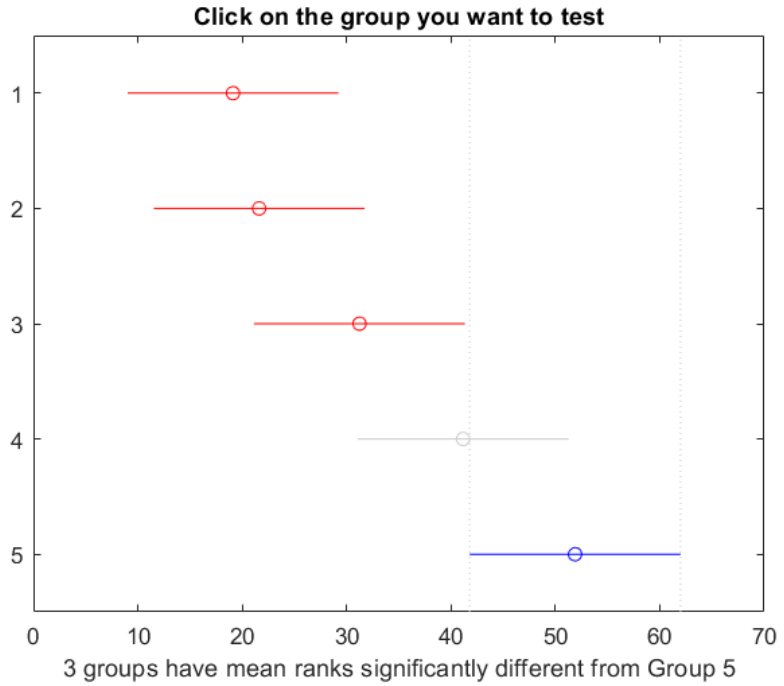


Figure 4: Post-hoc Tukey-Kramer Test Results.

6 Question 6: Manufacturing Concerns in Phase I Study

With the MTD/NOAEL measured and the starting dose selected, a phase I study was initiated in human participants. A single lot (801) of Pancomab8 was synthesized, but the yield was far too low to support the entire study. A second lot (802) failed due to an aseptic violation, and the third lot (803) was synthesized in conjunction with initiation of the study, with the intention of resupplying when lot 801 ran out. However, the third lot was found to produce severe liver toxicity at the second dose level in three patients, which was previously shown to be efficacious in tumor reduction. SPR was performed to assess binding kinetic data from n = 3 samples from each lot, and the DM1 content (i.e. the drug-antibody ratio/DAR), the percentage of DM1 as a free drug, average pharmacokinetic half-life, and peak ALT were assessed. These results (along with the derived equilibrium dissociation constant) are summarized in the table below. These results suggest a number of problems: the dissociation constant of the third lot is nearly

Table 6: Parameters from ADC Lots.

Lot	$k_a(10^5(Ms)^{-1})$	$k_d(10^{-4}s^{-1})$	$K_D(nM)$	DAR	$\%FreeDM1$	$t_{1/2,avg}(d)$	$ALT_{max}(U/L)$
801	5.6	1.3	0.23	3.4	<0.1	16	52
802	6.2	0.9	0.15	3.2	<0.1	N/A	N/A
803	3.8	3.0	0.79	6.8	0.9	7	1100

3.5 times higher than that of the first. This larger dissociation constant means that, for the same dose, a smaller fraction of the drug will bind to the EHR1 surface receptors. As a result, a smaller fraction will be internalized and will deliver DM1 to the cell interior to disrupt microtubule activity. The 2X higher DAR and higher fraction of free drug explain the severe toxicity and high peak ALT levels observed. The reduced half-life could be the result of the smaller dissociation constant, as lower internalization levels means more drug is available for clearance. One potential mechanism which could explain all of these observed factors is an incorrect amount of DM1 added during manufacturing. Excess drug could explain the increased free drug percentage if the amount of drug saturates the available antibody. This would also explain the toxicity observed in the ALT levels. If the DM1:antibody ratio is too large in the mixture, it is possible that excess DM1 will bind to the antibody as well, explaining the DAR increase. Though often not considered for smaller DARs, binding of drug to antibody molecules is capable of producing conformational changes which disrupt, for example, the domains which govern antibody binding and internalization. This could explain the larger dissociation constant. An added effect that is not directly explored here would be if the Fc binding domain was altered as well. This could impede antibody recycling, thereby further reducing efficacy of the conjugate.

If the cause is this simple, the solution is as well. More care must be taken in future fabrication attempts to ensure the correct amount of drug is added. This can be readily confirmed through quality control methods, including the use of SPR to confirm antibody binding kinetics. While a correct dissociation constant around $0.20nM$ does not guarantee the resultant product will work, it is likely that a batch with incorrect dissociation constant will not work correctly, as this would directly influence receptor binding and internalization and may serve as a proxy for correct DAR. Given the rather dramatic decrease in tumor growth rate over 25 days found in Figure 2 with the thioether linker, it would be unfortunate and ill-advised to abandon this project during clinical trials simply due to manufacturing difficulties, especially given that the proposed explanation has an extremely simple solution. Clearly, more care must be taken during manufacturing runs in the future, but this should not be enough of a deterrent to terminate the study. However, if correct values are used and the problem persists, then this must be re-evaluated.

References

[1] M. Pyzik, K. M. K. Sand, J. J. Hubbard, J. T. Andersen, I. Sandlie, and R. S. Blumberg, "The neonatal fc receptor (fcrn): A misnomer?," *Frontiers in Immunology*, vol. 10, p. 1540, 2019.

[2] A. Talkington and R. Durrett, "Estimating tumor growth rates in vivo," *Bulletin of mathematical biology*, vol. 77, no. 10, pp. 1934–1954, 2015.

[3] D. Prati, E. Taioli, A. Zanella, E. Della Torre, S. Butelli, E. Del Vecchio, L. Vianello, F. Zanuso, F. Mozzi, S. Milani, *et al.*, "Updated definitions of healthy ranges for serum alanine aminotransferase levels," *Annals of internal medicine*, vol. 137, no. 1, pp. 1–10, 2002.

[4] G. Shivaraj, D. Prakash, H. Vinayak, M. Avinash, V. Sonal, K. Shruthi, *et al.*, "A review on laboratory liver function tests.," *Pan African Medical Journal*, vol. 3, 2009.

[5] H.-K. Park, J.-W. Cho, B.-S. Lee, H. Park, J.-S. Han, M.-J. Yang, W.-J. Im, D.-Y. Park, W.-J. Kim, S.-C. Han, *et al.*, "Reference values of clinical pathology parameters in cynomolgus monkeys (macaca fascicularis) used in preclinical studies," *Laboratory animal research*, vol. 32, no. 2, pp. 79–86, 2016.

[6] R. Oh, T. R. Hustead, S. M. Ali, and M. W. Pantsari, "Mildly elevated liver transaminase levels: causes and evaluation," *American family physician*, vol. 96, no. 11, pp. 709–715, 2017.

7 Appendix

Script to analyze surface expression levels of three EHR receptors based on SPR data. Density of surface receptors of diseased and healthy tissues were divided and the mean ratio of diseased:healthy was obtained along with its standard deviation. As an alternative, the mean of each tissue type and receptor type were obtained and the means and standard deviations were divided.

```
Patients = [1001:1008];
EHR1 = [76,103,36,85,77,102,45,72;1872,2085,1620,1540,1963,1452,3125,842]';
EHR2 = [655,136,89,1488,1672,89,705,968;1489,855,1540,1650,1430,102,1945,780]';
EHR3 = [89,94,54,78,65,15,56,90;654,56,410,580,68,54,62,48]';
% molecules/um2

EHRavg = [mean(EHR1(:,1)),mean(EHR1(:,2));mean(EHR2(:,1)),mean(EHR2(:,2));mean(
    EHR3(:,1)),mean(EHR3(:,2))];
EHRstd = [std(EHR1(:,1)),std(EHR1(:,2));std(EHR2(:,1)),std(EHR2(:,2));std(EHR3
    (:,1)),std(EHR3(:,2))];

RelExpavgEHR = EHRavg(:,2)./EHRavg(:,1);
RelExpstdEHR = EHRstd(:,2)./EHRstd(:,1);

RelExp = [EHR1(:,2)./EHR1(:,1),EHR2(:,2)./EHR2(:,1),EHR3(:,2)./EHR3(:,1)];
RelExpavg = [mean(RelExp(:,1)),mean(RelExp(:,2)),mean(RelExp(:,3))];
stdavg = [std(RelExp(:,1)),std(RelExp(:,2)),std(RelExp(:,3))];
stdavgnorm = stdavg./RelExpavg;
```

Script for analyzing antibody binding kinetic data from SPR and phage display technology. Antibody dissociation constants are calculated for 8 patients from the forward and reverse reaction rate constants for three different candidate human antibodies. The mean for each antibody is calculated and 95% confidence intervals are estimated from a t-distribution.

```
Patients = [1001:1008];
N = length(Patients);

tscoreinv = tinv([0.025 0.975],N-1);
hAbka =
    [3.1,4.6,5.6,4.2,8.9,3.4,4.5,7.2;1.5,4.1,3.2,1.8,1.9,2.4,1.5,3.2;3.5,4.1,2.8,5.7,4.1,3

hAbkd =
    [1.5,1.6,1.3,1.8,3.0,1.2,1.1,1.8;1.9,2.5,3.6,4.9,8.7,4.7,4.8,7.6;9.8,7.3,12.8,9.7,13.0

%(Ms)^-1 and s^-1, respectively
hAbKD = hAbkd./hAbka;
%M

meanval = mean(hAbKD,2);
stdval = std(hAbKD,0,2);
CI = [meanval + tscoreinv.*stdval/sqrt(N)];
```

Script for pharmacokinetic analysis of the previously selected antibody and three versions with modified Fc domains to enhance FcRn binding and plasma recycling/half-life. Data is first plotted on a log-linear scale to examine for the presence of a distribution phase. Data is then fit to a one-compartment model using linear regression and the model fits are plotted on a linear scale with the experimental data. Volume of distribution, elimination rate constant, and elimination half-life are obtained from the fit parameters.

```
t = [0.5:0.5:4,5,6,7,8,10,12,14,16];
% days
Pancomab = [21 17 15 12 11 9 7 6 4 3 2 1 1 0.4 0.2 0.1;23 21 20 18 17 16 14 13
    11 10 8 7 5 4 3 2;24 23 22 21 20 19 18 18 16 15 14 13 11 9 7 6;24 24 23 22 22
     21 21 20 19 18 17 16 15 13 12 11];
```

```
% ug/mL
s = size(Pancomab);
weight = 5; %kg
Volperkg = 40; %mL/kg
Doseperkg = 1; %mg/kg
Vplasma = Volperkg * weight; %mL
Dose = 1000*Doseperkg * weight; %ug

colors = ['k';'b';'r';'g'];
figure;
hold on;
for i = 1:s(1)
   plot(t,log(Pancomab(i,:)),['o',colors(i)]);
end
legend({'Pancomab','PancomabF','PancomabG','PancomabH'});
xlabel('Time (days)');
ylabel('Natural Log of Plasma Concentration');
hold off;

for i = 1:s(1)
    fit(i,:) = polyfit(t,log(Pancomab(i,:)),1);
end

k = -fit(:,1);
t12 = log(2)./k;
Vd = Dose./exp(fit(:,2));

tfit = 0:0.5:16;
modelfit = (Dose./Vd).*exp(-k*tfit);
figure;
hold on;
for i = 1:s(1)
    plot(t,Pancomab(i,:),['o',colors(i)]);
end
for i = 1:s(1)
    plot(tfit,modelfit(i,:),colors(i));
end
legend({'Pancomab','PancomabF','PancomabG','PancomabH'});
xlabel('Time (days)');
ylabel('Plasma Concentration (\mu g/mL)');
```

Script for analysis of tumor growth data from unconjugated antibody and five different linkers conjugating the antibody to DM1 maytansinoid. Data is fit to an exponential model and tumor growth rate constants are obtained. Two-way ANOVA testing and a Tukey-Kramer post-hoc test are performed on the data to determine if time and linker produce statistically significant tumor growth behaviors.

```
t = [0 3 6 9 12 15 18 21];
time = 0:0.5:21;
%days
Pancomab = [190 240 330 440 600 820 NaN NaN;170 230 290 370 480 600 770 NaN;160
   220 270 350 430 500 640 780;180 210 270 310 370 450 520 640;170 200 240 280
   320 380 440 500;190 190 210 240 280 300 330 360]';
%mm^3
Pancomabnorm = Pancomab./Pancomab(1,:);
s = size(Pancomab);

for i = 1:s(2)
    valid(i) = nnz(~isnan(Pancomab(:,i)));
```

```
    fit(i,:) = polyfit(t(2:valid)',log(Pancomab(2:valid,i)),1);
end

figure;
modelfit = exp(fit(:,2)).*exp(fit(:,1)*time);
hold on;
r = fit(:,1);
V0 = exp(fit(:,2));
colors = ['k';'b';'r';'g';'y';'c'];
for i = 1:s(2)
    plot(time,modelfit(i,:),colors(i));
end
for i = 1:s(2)
    plot(t,Pancomab(:,i),['o',colors(i)]);
end
xlabel('Time (days)');
ylabel('Tumor volume (mm^3)');
legend({'Pancomab','Pancomab8a','Pancomab8b','Pancomab8c','Pancomab8d','
   Pancomab8e'});
hold off;
ylim([0 1000]);

[p,t,stats]=anova2(Pancomab(1:6,:));
c = multcompare(stats);
```

Script to analyze levels of liver alanine aminotransferase in the blood of monkeys to identify maximum tolerated dose. Raw data is plotted for five different doses and a control (with antibody and without toxin) and a Kruskal-Wallis rank test with Tukey-Kramer post-hoc is used to test the hypothesis that dose causes statistically significant differences in levels of liver enzyme. The linear regression coefficients for the region during which the drug is being administered is also computed to look for increases in ALT levels over time.

```
Doses = [2:2:8];
Week = [0:12];
ALT = [41 42 40 39 45 43 42 45 41 39 37 45 42;46 48 51 41 39 38 44 43 38 38 45
    49 38;44 44 49 48 42 56 41 52 54 56 41 40 42;45 48 65 58 68 56 75 52 89 54 42
     41 44;39 39 141 134 168 120 178 109 169 115 96 89 82]';
%U/L
[p,t,stats]=kruskalwallis(ALT);
results = multcompare(stats);
s = size(ALT);

figure;
hold on;
colors = ['k';'b';'r';'g';'c'];
for i = 1:s(2)
    plot(Week,ALT(:,i),['o',colors(i)]);
    mdl{i} = fitlm(Week(3:10)',ALT(3:10,i));
end
legend({'0 mg/kg','2 mg/kg','6 mg/kg','12 mg/kg','24 mg/kg'});
xlabel('Time (Weeks)');
ylabel('ALT Concentration (U/L)');
hold off;
```